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16. ABSTRACT

The chronic toxicity of mercury (Hg) to Daphnia magna was studied under flow-through and renewed static conditions. Concentrations of mercuric chloride ($HgCl_2$), methyl mercuric chloride (MMC) and phenyl mercuric acetate (PMA) in flow-through tests significantly affecting survival were 1.92, between 0.26 and 0.98, and 2.25 ug Hg/L, respectively. Concentrations of $HgCl_2$, MMC, and PMA significantly impairing young production ($P \leq 0.05$) were 0.72, 0.04, and 1.90 ug Hg/L, respectively. Body accumulation of mercury was greatly influenced by the chemical form of mercury in water. About nine times more mercury, added as MMC, was tolerated in daphnids at water concentrations permitting survival than was tolerated when added as $HgCl_2$. At about the same mercury concentration in water (~0.26 ug Hg/L) daphnids accumulated ~20 times more mercury when it was added as MMC than when it was added as $HgCl_2$. Mercury was rapidly accumulated in daphnids; however, 35 and 57% of the mercury added as MMC and $HgCl_2$, respectively, was lost when animals were placed in control water for four days following exposure. Different forms of mercury behaved quite differently in renewed-static and flow-through systems. The results also indicate the shortcomings of renewed-static tests with volatile and readily degradable compounds.

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Chronic Effects of Inorganic and Organic Mercury on *Daphnia magna*: Toxicity, Accumulation, and Loss

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Abstract. The chronic toxicity of mercury (Hg) to *Daphnia magna* was studied under flow-through and renewed static conditions. Concentrations of mercuric chloride ($HgCl_2$), methyl mercuric chloride (MMC) and phenyl mercuric acetate (PMA) in flow-through tests significantly affecting survival were 1.92, between 0.26 and 0.98, and 2.25 μg Hg/L, respectively. Concentrations of $HgCl_2$, MMC, and PMA significantly impairing young production ($P \leq 0.05$) were 0.72, 0.04, and 1.90 μg Hg/L, respectively. Body accumulation of mercury was greatly influenced by the chemical form of mercury in the water. About nine times more mercury, added as MMC, was tolerated in daphnids at water concentrations permitting survival than was tolerated when added as $HgCl_2$. At about the same mercury concentration in water (~0.26 μg Hg/L) daphnids accumulated ~20 times more mercury when it was added as MMC than when it was added as $HgCl_2$. Mercury was rapidly accumulated in daphnids; however, 35 and 57% of the mercury added as MMC and $HgCl_2$, respectively, was lost when animals were placed in control water for four days following exposure. Different forms of mercury behaved quite differently in renewed-static and flow-through systems. The results also indicate the shortcomings of renewed-static tests with volatile and readily degradable compounds.

The discovery of high mercury concentrations in fish has resulted in additional research and a better understanding of mercury forms in sediment and water, bacterial conversions of mercury, toxicity to aquatic organisms, uptake and loss by aquatic organisms and bioaccumulation through the food chain. Many bioassay studies have been conducted to determine the acute effects of various compounds of mercury to aquatic life; there have also been studies of chronic effects on fish (Olson *et al.* 1975; McKim *et al.* 1976; and Snarski and Olson 1981), *Daphnia* (Biesinger and Christensen 1972), and zooplankton communities in enclosures (Beers *et al.* 1977; Brown and Parsons 1978; and Marshall *et al.* 1981). Other work has included bioconcentration (Olson *et al.* 1975; Phillips and Buhler 1980; McKim *et al.* 1976; and Boudou *et al.* 1979) and loss studies (Burrows and Krenkel 1973).

The present study with *Daphnia magna* determined the chronic effects of one inorganic and two organic mercury compounds on survival and reproduction; plus the determination of the relative uptake, body accumulation and loss of inorganic and organic mercury. In addition, behavioral studies were made of the different forms of mercury in renewed-static and flow-through systems.

Materials and Methods

Testing Methods

Daphnia magna, cultured at the Environmental Research Laboratory in Duluth, MN, were used for testing under flow-through and renewed static conditions. For flow-through tests, five daphnids 12 ± 12-hr-old were placed in battery jars containing 1.5 L of water. For each test, four replicate test chambers with a total of 20 animals were used for each experimental treatment and control. Test chambers were randomly placed on a shelf in a constant temperature room (18 ± 1°C). After both the first and second week of exposure, daphnids were transferred into jars with a medicine dropper and the complete test apparatus was thoroughly cleaned with nitric acid to kill bacteria, fungi, and algae. Survival of the animals added initially was noted both weekly and at the end of the three-week exposure period. During the test, the young produced each week were counted and either discarded or set aside for mercury analysis.

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A combination of Duro-Test[1] (Optima FS) and wide spectrum Gro-Lux fluorescent bulbs provided ~115 lumens of light at the air-water interface. A 16-hr photoperiod was controlled automatically.

A system similar to the one described by Brungs and Mount (1970) was used to deliver 2 L of water to two mixing chambers where suspensions of trout fry starter granules and powdered dried grass (Biesinger and Christensen 1972) were added, using a Mariotte bottle apparatus which delivered 0.5 mg dry weight of the food in a water suspension per L of water to a mixing chamber each time the diluter cycled. After the food addition, water flowed from the mixing chambers into proportional diluters (Mount and Brungs 1967) where toxicants were added with syringe injectors. Fifty ml of water containing food were added to each control and exposure chamber every time the diluter cycled. The flow rate was regulated to three tank volumes per day. Static renewal tests were conducted in a manner similar to flow-through tests except that 250 ml beakers, each containing 200 ml of test or control solutions, were used as test containers and the medium was renewed manually once a week. Four replicate test chambers with a total of 20 animals were used for each experimental treatment and control. Details of static renewal tests were described by Biesinger and Christensen (1972).

Two tests were made with *Daphnia* at different times to determine the loss of mercury, added as $HgCl_2$ and as MMC, by exposing 40 animals (12 ± 12-hr-old) to each experimental and control condition for four days in standing jars containing 1.5 L of solution. One-half of the animals were then prepared for tissue analysis, and the remainder were placed in flowing water not containing mercury for an additional four days. At this time, all remaining animals were prepared for tissue analysis. This test was conducted for eight days, because daphnids start reproducing on the ninth day which would have confounded the results.

Chemical Measurements

Unfiltered Lake Superior water was used for all testing. The chemical characteristics of this water during the test period were a pH of 7.6 (7.3-8.0), a total hardness of 45 (±1.26) mg/L (as $CaCO_3$) and an alkalinity of 42 (±1.19) mg/L (as $CaCO_3$). Other chemical characteristics are given in Biesinger and Christensen (1972).

Reagent grade mercuric chloride ($HgCl_2$), methyl mercuric chloride (CH_3HgCl) and phenylmercuric acetate ($CH_3COOHgC_6H_5$) were used for testing.

Water samples were collected from the flow-through system for analysis. The static renewal system was sampled at the beginning and at the end of each week by transferring *Daphnia* and pouring the water sample from the beaker before acidification. The samples were analyzed for total mercury by the flameless atomic absorption procedure described by Olson *et al.* (1975). Organic mercury was analyzed by the procedure given in Olson *et al.* (1975). Precision and accuracy were checked by adding 1.00 and 0.10 μg Hg/L to the control water and determining the recovery. Recovery from water samples and reagent blanks was analyzed each time samples were analyzed; recovery was 96.6 ± 6.7%.

For bioaccumulation flow-through tests, daphnids were prepared for Hg residue analysis by straining the young animals at the end of the second week and the young and adults at the end of the third week (no young were produced during the first week) from the medium onto a stainless steel screen (250 mesh), placing them in small plastic containers with forceps and drying them in a desiccator at 18°C to constant weight. For each test, all animals from each concentration were pooled for analysis. After drying, they were weighed and placed in 125 ml flat bottom boiling flasks for mercury analysis. Tissues were analyzed for mercury according to the method of Olson *et al.* (1975) with a modification in volume due to the small sample size (1 to 50 mg dry weight). Calculations of mercury in *Daphnia* are based on dry weight measurements (~10% of wet weight) and are expressed as μg Hg/g of tissue and also as μg Hg/animal.

Statistical Analysis

The effects on adult and young production were assessed using a $\sqrt{x} + \sqrt{x+1}$ (x = number of adults surviving or young produced per beaker) transformation to allow the use of zeros, and an analysis of variance (Dunnett's test, one-tailed, $P \leq 0.05$). Mercury concentrations in the flow-through systems are expressed as means and standard deviations. Mercury was lost in renewed static tests between the time when new test solutions were prepared and after one week. A series of measurements were made to determine mercury loss after 1, 2, 4, 8, and 24 hr and thereafter daily for one week to determine the disappearance rate. The following exponential decay formula was employed to obtain average concentrations with time:

$$A = \frac{Y(o)[1 - e^{-t\lambda}]}{t\lambda}$$

where

A = average concentration,
$Y(o)$ = concentration at time o,
λ = the instantaneous loss rate or $(\ln_e 2)/\lambda$ = half life, and
t = time in hours.

The concentrations thus obtained are expressed as means and standard deviations.

Results

Chronic Toxicity

The compounds of mercury tested, mercury concentrations tested, percent survival, and average number of young per test chamber under flow-through and renewed static test conditions are given in Tables 1 and 2. Concentrations of mercury affecting survival in μg Hg/L were: 2.70 and > 0.26 for $HgCl_2$ and MMC, respectively, under flow-through conditions (Table 1); and 3.53, 0.87, and 1.90 for $HgCl_2$, MMC and PMA, respectively, under renewed static conditions (Table 2). Test results are summarized in Table 3 as Hg concentrations significantly affecting survival and as Hg concentrations significantly reducing young production compared with the controls. In flow-through tests, young production was

[1] The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used only for identification

Table 1. Chronic toxicity and bioconcentration of mercury by *Daphnia magna* under flow-through water conditions

Toxicant	Water concentration of mercury (μg/L ± SD)	Adult 21 day survival (%)	Average number of young per test container ± SD	Mean concentration of mercury in tissue (μg/g)
Mercuric chloride[a]	Control <0.01 (7)[c]	100	286 ± 68	1.26 (2)
	0.36 ± 0.12 (9)	95	291 ± 118	8.59 (2)
	0.72 ± 0.30 (10)	98	257 ± 100	15.26 (2)
	1.28 ± 0.45 (9)	92	194 ± 59*	23.28 (2)
	2.70 ± 0.94 (3)	0**	0**	—
Methyl mercuric chloride[b]	Control <0.01 (5)	90	213 ± 57	0.92 (1)
	0.04 ± 0.01 (5)	85	138 ± 19*	16.42 (1)
	0.07 ± 0.02 (5)	95	80 ± 16**	46.67 (1)
	0.13 ± 0.06 (5)	95	78 ± 24**	75.67 (1)
	0.26 ± 0.13 (4)	65	2 ± 3**	183.75 (1)

[a] Two tests
[b] One test
[c] Number of measurements
* Significant difference ($P \leq 0.05$)
** Significant difference ($P \leq 0.01$)

Table 2. Chronic toxicity of mercury to *Daphnia magna* under renewed static conditions

Toxicant	Nominal Hg concentration	Water concentration of Hg (μg/L ± SD)	Number animals tested at each concentration	Adult 21 day survival (%)	Average number of young per test container ± SD
Mercuric chloride	Control	<0.1 (12)[a]	180 (9)[b]	95	68 ± 25
	0.85	0.43 ± 0.01 (14)	80 (4)	81	67 ± 20
	1.70	0.91 ± 0.07 (12)	80 (4)	91	73 ± 37
	3.40	1.82 ± 0.18 (12)	180 (9)	89	53 ± 23*
	6.80	3.53 ± 0.29 (16)	180 (9)	60**	34 ± 22**
	13.60	5.31 ± — (2)	20 (1)	0**	0**
Methyl mercuric chloride	Control	<0.1 (8)	80 (4)	96	115 ± 54
	0.25	0.17 ± 0.04 (4)	80 (4)	99	114 ± 57
	0.50	0.28 ± 0.04 (4)	80 (4)	95	116 ± 50
	1.00	0.52 ± 0.04 (4)	80 (4)	95	121 ± 30
	2.00	0.87 ± 0.04 (12)	80 (4)	89*	111 ± 46
	4.00	1.14 ± 0.10 (4)	80 (4)	0**	3 ± 9**
Phenyl mercuric acetate	Control	<0.1 (6)	35 (2)	94	81 ± 53
	0.63	0.35 ± — (2)	15 (1)	100	119 ± 16
	1.25	0.54 ± — (2)	15 (1)	87	116 ± 32
	2.50	1.12 ± 0.07 (10)	35 (2)	94	84 ± 42
	5.00	1.90 ± 0.20 (10)	35 (2)	74*	56 ± 23
	10.00	3.20 ± — (2)	35 (2)	3**	2**

[a] Number of measurements
[b] Number of tests (Note: One test with PMA was run with 3 replicates, all other tests were run with 4 replicates)
* Significant difference ($P \leq 0.05$)
** Significant difference ($P \leq 0.01$)

significantly reduced, (Table 3), by 1.28 μg Hg/L as $HgCl_2$. All concentrations of mercury as MMC tested in the flow-through system (the lowest tested was 0.04 μg Hg/L) caused a significant decrease in young production. The lowest concentrations significantly impairing young production in renewed static tests were 1.82, 1.14, and 3.20 μg Hg/L for $HgCl_2$, MMC and PMA, respectively (Table 3). In renewed static tests, inorganic mercury significantly impaired reproduction at concentrations lower than significant effects on survival. In contrast to inorganic mercury, organic mercury (MMC

Table 3. Chronic toxicity of three compounds of mercury to *Daphnia magna* expressed as μg Hg/L[a]

Toxicant	Type of test	Concentration significantly affecting survival	Young production	
			Significant impairment	No significant impairment
Mercuric chloride ($HgCl_2$)	Flow-through	2.70	1.28	0.72
	Renewed static	3.53	1.82	0.91
Methyl mercuric chloride (MMC)	Flow-through	>0.26	0.04	<0.04
	Renewed static	0.87	1.14	0.87
Phenyl mercuric acetate (PMA)	Flow-through	—	—	—
	Renewed static	1.90	3.20	1.90

[a] See Tables 1 and 2 for significance

and PMA) concentrations significantly impairing reproduction were higher than significant corresponding effects on survival (Table 3).

Bioaccumulation

In flow-through tests, the uptake of mercury by daphnids was greatly influenced by the form of mercury in the water (Table 1 and Figure 1). When mercury was added as $HgCl_2$, ~20 times less mercury was taken up by daphnids at a water concentration of ~0.26 μg Hg/L than when added as MMC in three week experiments (Figure 1). The uptake of both inorganic (Hg^{++}) and organic (CH_3Hg^+) forms of mercury by daphnids was linear and highly correlated (r = 0.996) with mercury concentrations in the water.

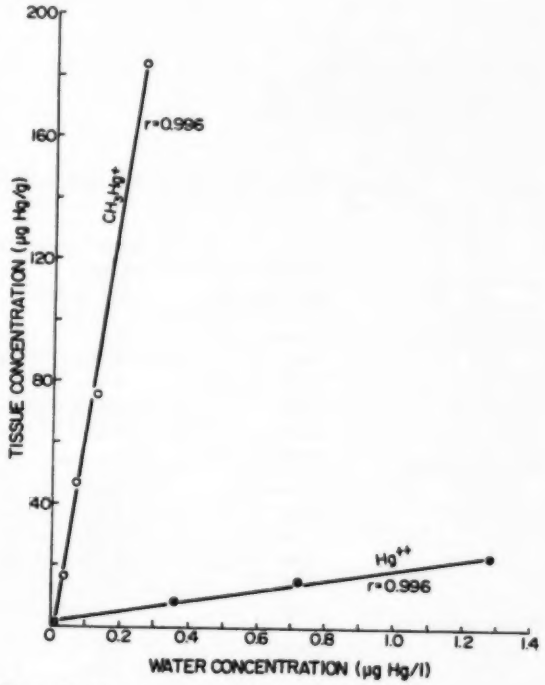

Fig. 1. Residues of mercury in *Daphnia magna* after exposure to methyl mercuric chloride (CH_3Hg^+) and mercuric chloride (Hg^{++}) for three weeks

Loss

The loss of Hg in four days in water without mercury added after allowing four days for Hg uptake is given in Table 4. The percent loss in Hg-free water was greater for inorganic mercury (57%) than for organic mercury (35%).

Discussion and Conclusions

Mercury added as $HgCl_2$, PMA, or MMC is toxic to *Daphnia magna* in low μg/liter concentrations. Mercury added as MMC was much more toxic than when added as $HgCl_2$ in flow-through tests. In renewed static tests, the difference in toxicity was not as pronounced. In flow-through tests, mercury added as MMC was ~3 times more toxic as noted by adult survival and ~28 times more toxic as noted by impairment of young production than in standing tests. By contrast, mercury added as $HgCl_2$ in flowing and renewed static tests produced differences that were less than 2-fold (Table 3). Additional analyses of MMC and $HgCl_2$ in renewed static tests showed that only 20 to 30% of the total toxicant remained in the water phase after seven days. Approximately 25% of the mercury was adsorbed to the glassware, 20 to 25% volatilized, while the remainder was taken up by the organisms. When MMC was the form initially added, 20 to 30% in the water phase at the end of the test had been con-

Table 4. Mercury concentrations in *Daphnia magna* after four days' exposure under renewed static conditions and subsequent loss after four days in Hg-free water under flow-through conditions

Compound	Test number	Mercury added μg/L	Mercury in ng Hg/animal: After 4 days exposure in mercury water	Mercury in ng Hg/animal: After 4 additional days in clean water	% loss per test	Mean % loss
Control	I	0	<0.2	<0.2	0	0
	II	0	0.1	0.1	0	
Mercuric chloride ($HgCl_2$)	I	3.4	0.7	0.3	57	57
	II	3.4	0.7	0.3	57	
Methyl mercuric chloride (MMC)	I	2.1	1.3	0.8	38	35
	II	2.1	1.0	0.7	30	

verted to ~90% inorganic mercury. Therefore, daphnids were exposed to considerably less organic mercury in renewed static systems.

The breakdown of organic mercury by bacteria is discussed by Spangler *et al.* (1973a, b), who concluded that bacteria are important in controlling the amount of MMC in the environment. We assume bacteria were largely responsible for the results observed here. Mercury added as PMA was tested under renewed static conditions only and only total mercury was measured. Consequently, the probability of rapid breakdown of PMA in water (Matsumura *et al.* 1971) and in tissue (Berlin and Ullberg 1963; Caton *et al.* 1971) made it impossible to resolve the relative toxicity of mercury as PMA from our experiments.

Inorganic mercury water concentrations were severely diminished in static test containers when measured soon after addition, at subsequent hourly intervals, and then daily up to one week. This loss of mercury by volatilization and adsorption has not often been reported by investigators and hence mercury concentrations given in the literature should be considered less than those reported, unless test solution measurements are included. For example, Biesinger and Christensen (1972) reported a 16% reproductive impairment in daphnids at an added but not analytically determined concentration of 3.4 μg Hg/L added as $HgCl_2$. Because of mercury loss, the average concentration in that study, based on measurements reported here, was probably closer to 1.8 μg Hg/L.

The results of this study show some of the advantages of using flow-through water test systems. Mercury loss from the water in renewed static tests occurred by both biological activity (bacteria, algae uptake, and the test animals themselves) and physical effects (adsorption and volatilization). While some loss also occurred in flow-through systems, concentrations remained fairly stable throughout the test period because of constant mercury addition. However, Olson *et al.* (1975) reported breakdown of MMC in their flowing tests as algae and bacteria were purposely left in test containers to provide food for fathead minnows. McKim *et al.* (1976) found very little (8 to 10%) breakdown in their test chambers as bacteria and algae were not allowed to build up.

The inorganic mercury concentration of 1.28 (flow through) to 1.82 (renewed static) μg Hg/l affecting young production is in close agreement with results reported by others working with zooplankton. Beers *et al.* (1977) reported that 5 μg Hg/L had detrimental effects on marine zooplankton. Brown and Parsons (1978) found that after 72 days marine zooplankton counts in enclosed environmental chambers containing 5 μg Hg/L (added as $HgCl_2$) were one-fourth of those exposed to 1 μg Hg/L, or controls. They reported that mercury replaced copper and zinc in metalloenzymes at concentrations higher than 1 μg Hg/L, and that adverse effects from mercury may be because of inhibition or alteration in enzyme function. A significant reduction in the number of *Daphnia galeata mendota* from the addition of 2 μg Hg/L (added as $HgCl_2$) in *in situ* experiments in carboys was observed by Marshall *et al.* (1981). They reported a significant reduction of zooplankton density, community similiarity and species diversity at a concentration of 4 μg Hg/L. Concentrations of mercury added as MMC chronically affecting zooplankton were not found in the literature.

Water concentrations of mercury as MMC were more toxic than mercury as $HgCl_2$ as determined by effects on survival and young production. However, the amount of mercury in tissue which *Daphnia* tolerated without mortality was about nine times greater when added as MMC than when added as $HgCl_2$ (Figure 1). The animals took up more mercury when added as MMC; however they tolerated less in

the water. As aquatic organisms concentrate mercury from the aquatic food chain (Hannerz 1968; Jernelov and Lann 1971; Phillips and Buhler 1980; and Boudou *et al.* 1979), the high mercury concentrations in *Daphnia* reported here would be expected to increase the mercury concentrations in organisms eating them, and thus there is cause for concern.

The uptake of mercury by daphnids in four days (Table 4) was rapid. The subsequent loss, which was greater for mercury as $HgCl_2$ than for mercury as MMC, was surprising considering that various studies report slow mercury loss in extended time periods in clean water following mercury exposure (Jarvenpaa *et al.* 1970; Lockhart *et al.* 1972; Burrows and Krenkel 1973; and McKim *et al.* 1976). The reduction found in this study is probably explained in part by the breakdown of organic mercury, by loss through the two to three molts which the young animals underwent during this period and by loss through the gastrointestinal tract.

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